

We claim:

1. A process for the production of  $\beta$ -carotenoids by culturing genetically modified plants which, in comparison to the wild-type, have an increased  $\beta$ -cyclase activity  
5 in plant tissues comprising photosynthetically inactive plastids, and the increased  $\beta$ -cyclase activity is caused by a  $\beta$ -cyclase comprising the amino acid sequence SEQ. ID. NO. 2 or a sequence derived from this sequence by substitution, insertion or deletion of amino acids, which has an identity of at least 60% at the amino acid level with the sequence SEQ. ID. NO. 2, with the proviso that tomato is excluded  
10 as a plant.
2. A process as claimed in claim 1, wherein, for the increase in the  $\beta$ -cyclase activity the gene expression of a nucleic acid encoding a  $\beta$ -cyclase comprising the amino acid sequence SEQ. ID. NO. 2 or a sequence derived from this sequence by  
15 substitution, insertion or deletion of amino acids, which has an identity of at least 60% at the amino acid level with the sequence SEQ. ID. NO. 2, is increased compared to the wild-type.
3. A process as claimed in claim 2, wherein, for the increase in the gene expression,  
20 nucleic acids are introduced into the plants which encode  $\beta$ -cyclases comprising the amino acid sequence SEQ. ID. NO. 2 or a sequence derived from this sequence by substitution, insertion or deletion of amino acids, which has an identity of at least 60% at the amino acid level with the sequence SEQ. ID. NO. 2.
- 25 4. A process as claimed in one of claims 1 to 3, wherein nucleic acids comprising the sequence SEQ. ID. NO. 1 are introduced.
5. A process as claimed in one of claims 1 to 4, wherein the expression of the  $\beta$ -cyclase takes place under the control of a promoter which guarantees the  
30 expression of the  $\beta$ -cyclase in the plant tissues comprising photosynthetically inactive plastids.
6. A process as claimed in one of claims 1 to 5, wherein genetically modified plants are used which have the highest expression rate of the  $\beta$ -cyclase in plant tissues

comprising photosynthetically inactive plastids.

7. A process as claimed in claim 6, wherein the expression of the  $\beta$ -cyclase takes place under the control of a promoter specific for the plant tissue.
- 5 8. A process as claimed in one of claims 1 to 7, wherein the plants additionally have an increased hydroxylase activity, compared to the wild-type.
- 10 9. A process as claimed in claim 8, wherein, for the additional increase in the hydroxylase activity, the gene expression of a nucleic acid encoding a hydroxylase is increased compared to the wild-type.
- 15 10. A process as claimed in claim 9, wherein, for the increase in the gene expression, a nucleic acid encoding a hydroxylase is introduced into the plant.
- 20 11. A process as claimed in claim 10, wherein, as a nucleic acid encoding a hydroxylase, nucleic acids are introduced which encode a hydroxylase comprising the amino acid sequence SEQ ID NO: 9 or a sequence derived from this sequence by substitution, insertion or deletion of amino acids, which have an identity of at least 20 % at the amino acid level with the sequence SEQ ID NO: 9.
- 25 12. A process as claimed in claim 11, wherein nucleic acids comprising the sequence SEQ ID NO: 8 are introduced.
- 30 13. A process as claimed in one of claims 1 to 12, wherein genetically modified plants are used which have the highest expression rate of the hydroxylase in plant tissues comprising photosynthetically inactive plastids.
14. A process as claimed in claim 13, wherein the expression of the hydroxylase takes place under the control of a promoter specific for the plant tissue.
15. A process as claimed in one of claims 1 to 14, wherein the plants, compared to the wild-type, additionally have a reduced activity of at least one of the activities selected from the group consisting of  $\epsilon$ -cyclase activity and endogenous

$\beta$ -hydroxylase activity.

16. A process as claimed in claim 15, wherein the reduction of the  $\epsilon$ -cyclase activity and/or of the endogenous  $\beta$ -hydroxylase activity in plants is achieved by at least one of the following processes:
- a) introduction of at least one double-stranded  $\epsilon$ -cyclase ribonucleic acid sequence and/or endogenous  $\beta$ -hydroxylase ribonucleic acid sequence or an expression cassette or expression cassettes guaranteeing their expression in plants,
- b) introduction of at least one  $\epsilon$ -cyclase antisense ribonucleic acid sequence and/or endogenous  $\beta$ -hydroxylase antisense ribonucleic acid sequence or an expression cassette or expression cassettes guaranteeing their expression in plants,
- c) introduction of at least one  $\epsilon$ -cyclase antisense ribonucleic acid sequence and/or endogenous  $\beta$ -hydroxylase antisense ribonucleic acid sequence in each case combined with a ribozyme or an expression cassette or expression cassettes guaranteeing their expression in plants,
- d) introduction of at least one  $\epsilon$ -cyclase sense ribonucleic acid sequence and/or endogenous  $\beta$ -hydroxylase sense ribonucleic acid sequence for the induction of a cosuppression or of an expression cassette or expression cassettes guaranteeing their expression in plants,
- e) introduction of at least one DNA- or protein-binding factor against an  $\epsilon$ -cyclase gene, RNA or protein and/or endogenous  $\beta$ -hydroxylase gene, RNA or protein or an expression cassette or expression cassettes guaranteeing its expression in plants,
- f) introduction of at least one viral nucleic acid sequence or nucleic acid sequences bringing about the  $\epsilon$ -cyclase RNA and/or endogenous  $\beta$ -hydroxylase RNA degradation or an expression cassette or expression cassettes guaranteeing their expression in plants,

- g) introduction of at least one construct for the production of an insertion, deletion, inversion or mutation in an  $\epsilon$ -cyclase gene and/or endogenous  $\beta$ -hydroxylase gene in plants.

5 17. A process as claimed in claim 16, embodiment a), wherein an RNA is introduced into the plant, which has a region having double-strand structure and in this region comprises a nucleic acid sequence which

10 a) is identical with at least one part of the plant-intrinsic  $\epsilon$ -cyclase transcript and/or

b) is identical with at least one part of the plant-intrinsic  $\epsilon$ -cyclase promoter sequence.

15 18. A process as claimed in claim 17, wherein the region having double-strand structure comprises a nucleic acid sequence which is identical with at least one part of the plant-intrinsic  $\epsilon$ -cyclase transcript and comprises the 5' end or the 3' end of the plant-intrinsic nucleic acid encoding an  $\epsilon$ -cyclase.

20 19. A process as claimed in claim 16, embodiment a), wherein an RNA is introduced into the plant which has a region having double-strand structure and in this region comprises a nucleic acid sequence which

25 a) is identical with at least one part of the plant-intrinsic, endogenous  $\beta$ -hydroxylase transcript and/or

b) is identical with at least one part of the plant-intrinsic, endogenous  $\beta$ -hydroxylase promoter sequence.

30 20. A process as claimed in claim 19, wherein the region having double-strand structure contains a nucleic acid sequence which is identical with at least one part of the plant-intrinsic, endogenous  $\beta$ -hydroxylase transcript and comprises the 5' end or the 3' end of the plant-intrinsic nucleic acid encoding an endogenous  $\beta$ -hydroxylase.

35 21. A process as claimed in one of claims 15 to 20, wherein genetically modified plants are used which have the lowest expression rate of an  $\epsilon$ -cyclase and/or

endogenous  $\beta$ -hydroxylase in plant tissues comprising photosynthetically inactive plastids.

22. A process as claimed in claim 21, wherein the transcription of the double-stranded  
5 ribonucleic acid sequence as set forth in claim 16, embodiment a) and/or the  
antisense sequences as set forth in claim 16, embodiment b) takes place under the  
control of a promoter which is specific for the plant tissues comprising  
photosynthetically inactive plastids.
- 10 23. A process as claimed in one of claims 1 to 22, wherein, after culturing, the  
genetically modified plants are harvested and subsequently the  $\beta$ -carotenoids are  
isolated from the plants or the plant tissues comprising photosynthetically inactive  
plastids.
- 15 24. A process as claimed in one of claims 1 to 23, wherein the plant tissue comprising  
photosynthetically inactive plastids is selected from the group consisting of flower,  
fruit and tuber.
- 20 25. A process as claimed in one of claims 1 to 24, wherein the genetically modified  
plant used which, in comparison to the wild-type, has an increased  $\beta$ -cyclase  
activity in flowers, is a plant selected from the families Ranunculaceae,  
Berberidaceae, Papaveraceae, Cannabaceae, Rosaceae, Fabaceae, Linaceae,  
Vitaceae, Brassiceae, Cucurbitaceae, Primulaceae, Caryophyllaceae,  
Amaranthaceae, Gentianaceae, Geraniaceae, Caprifoliaceae, Oleaceae,  
25 Tropaeolaceae, Solanaceae, Scrophulariaceae, Asteraceae, Liliaceae,  
Amaryllidaceae, Poaceae, Orchidaceae, Malvaceae, Illiaceae or Lamiaceae.
26. A process as claimed in claim 25, wherein the plant used is a plant selected from  
the plant genera Marigold, Tagetes erecta, Tagetes patula, Acacia, Aconitum,  
30 Adonis, Arnica, Aquilegia, Aster, Astragalus, Bignonia, Calendula, Calendula  
officinalis, Caltha, Campanula, Canna, Centaurea, Cheiranthus, Chrysanthemum,  
Citrus, Crepis, Crocus, Curcubita, Cytisus, Delonia, Delphinium, Dianthus,  
Dimorphotheca, Doronicum, Eschscholtzia, Forsythia, Fremontia, Gazania,  
Gelsemium, Genista, Gentiana, Geranium, Gerbera, Geum, Grevillea, Helenium,  
35 Helianthus, Hepatica, Heracleum, Hisbiscus, Heliopsis, Hypericum, Hypochoeris,

Impatiens, Iris, Jacaranda, Kerria, Laburnum, Lathyrus, Leontodon, Lilium, Linum, Lotus, Lysimachia, Maratia, Medicago, Mimulus, Narcissus, Oenothera, Osmanthus, Petunia, Photinia, Physalis, Phyteuma, Potentilla, Pyracantha, Ranunculus, Rhododendron, Rosa, Rudbeckia, Senecio, Silene, Silphium, Sinapsis, Solanum tuberosum, Sorbus, Spartium, Tecoma, Torenia, Tragopogon, Trollius, Tropaeolum, Tulipa, Tussilago, Ulex, Viola or Zinnia.

27. A process as claimed in one of claims 1 to 24, wherein the a genetically modified plant used which, in comparison to the wild-type, has an increased  $\beta$ -cyclase activity in fruits, is a plant selected from the plant genera Actinophloeus, Aglaeonema, Ananas, Arbutus, Archontophoenix, Area, Aronia, Asparagus, Avocado, Attalea, Berberis, Bixia, Brachychilum, Bryonia, Caliptocalix, Capsicum, Carica, Celastrus, Citrullus, Citrus, Convallaria, Cotoneaster, Crataegus, Cucumis, Cucurbita, Cuscuta, Cycas, Cyphomandra, Dioscorea, Diospyrus, Dura, Elaeagnus, Elaeis, Erythroxylon, Euonymus, Erbse, Ficus, Fortunella, Fragaria, Gardinia, Gonocaryum, Gossypium, Guava, Guilielma, Hibiscus, Hippophaea, Iris, Kiwi, Lathyrus, Lonicera, Luffa, Lycium, Mais, Malpighia, Mangifera, Mormodica, Murraya, Musa, Nenga, Orange, Palisota, Pandanus, Passiflora, Persea, Physalis, Prunus, Ptychandra, Punica, Pyracantha, Pyrus, Ribes, Rosa, Rubus, Sabal, Sambucus, Seaforita, Shepherdia, Solanum, Sorbus, Synaspadix, Tabernae, Tamus, Taxus, Trichosanthes, Triphasia, Vaccinium, Viburnum, Vignia, Vitis or Zucchini.
28. A process as claimed in one of claims 1 to 24, wherein the genetically modified plant used which, in comparison to the wild-type, has an increased  $\beta$ -cyclase activity in tubers, is Solanum tuberosum.
29. A process as claimed in one of claims 1 to 28, wherein the  $\beta$ -carotenoids are selected from the group consisting of  $\beta$ -carotene,  $\beta$ -cryptoxanthin, zeaxanthin, antheraxanthin, violaxanthin and neoxanthin.
30. A genetically modified plant, the genetic modification increasing the activity of a  $\beta$ -cyclase in plant parts comprising photosynthetically inactive plastids, compared to the wild-type, and the increased  $\beta$ -cyclase activity being caused by a  $\beta$ -cyclase comprising the amino acid sequence SEQ. ID. NO. 2 or a sequence derived from

this sequence by substitution, insertion or deletion of amino acids, which has an identity of at least 60% at the amino acid level with the sequence SEQ. ID. NO. 2.

- 5 31. A genetically modified plant as claimed in claim 30, wherein the increase in the  $\beta$ -cyclase activity is brought about by an increase in the gene expression of a nucleic acid encoding a  $\beta$ -cyclase comprising the amino acid sequence SEQ. ID. NO. 2 or a sequence derived from this sequence by substitution, insertion or deletion of amino acids, which has an identity of at least 60% at the amino acid level with the sequence SEQ. ID. NO. 2, compared to the wild-type.
- 10 32. A genetically modified plant as claimed in claim 31, wherein, for the increase in the gene expression, nucleic acids are introduced into the plant which encode  $\beta$ -cyclases comprising the amino acid sequence SEQ. ID. NO. 2 or a sequence derived from this sequence by substitution, insertion or deletion of amino acids,
- 15 33. A genetically modified plant comprising at least one nucleic acid encoding a  $\beta$ -cyclase comprising the amino acid sequence SEQ. ID. NO. 2 or a sequence derived from this sequence by substitution, insertion or deletion of amino acids, which has an identity of at least 60% at the amino acid level with the sequence SEQ. ID. NO. 2, with the proviso that tomato is excluded.
- 20 34. A genetically modified plant as claimed in one of claims 30 to 33, wherein the genetic modification additionally increases the hydroxylase activity compared to the wild-type.
- 25 35. A genetically modified plant as claimed in one of claims 30 to 34, wherein the genetic modification additionally reduces at least one of the activities selected from the group consisting of  $\epsilon$ -cyclase activity and endogenous  $\beta$ -hydroxylase activity compared to the wild-type.
- 30 36. A genetically modified plant as claimed in one of claims 30 to 35, wherein the plant tissue comprising photosynthetically inactive plastids is selected from the group

consisting of flower, fruit and tuber.

37. A genetically modified plant as claimed in one of claims 30 to 36, wherein the genetically modified plant which, in comparison to the wild-type, has an increased  $\beta$ -cyclase activity in flowers is selected from the families Ranunculaceae, Berberidaceae, Papaveraceae, Cannabaceae, Rosaceae, Fabaceae, Linaceae, Vitaceae, Brassicaceae, Cucurbitaceae, Primulaceae, Caryophyllaceae, Amaranthaceae, Gentianaceae, Geraniaceae, Caprifoliaceae, Oleaceae, Tropaeolaceae, Solanaceae, Scrophulariaceae, Asteraceae, Liliaceae, Amaryllidaceae, Poaceae, Orchidaceae, Malvaceae, Illiaceae or Lamiaceae.
38. A genetically modified plant as claimed in claim 37, wherein the plant is selected from the plant genera Marigold, Tagetes erecta, Tagetes patula, Acacia, Aconitum, Adonis, Arnica, Aquilegia, Aster, Astragalus, Bignonia, Calendula, Calendula officinalis, Caltha, Campanula, Canna, Centaurea, Cheiranthus, Chrysanthemum, Citrus, Crepis, Crocus, Curcubita, Cytisus, Delonia, Delphinium, Dianthus, Dimorphotheca, Doronicum, Eschscholtzia, Forsythia, Fremontia, Gazania, Gelsemium, Genista, Gentiana, Geranium, Gerbera, Geum, Grevillea, Helenium, Helianthus, Hepatica, Heracleum, Hibiscus, Heliopsis, Hypericum, Hypochoeris, Impatiens, Iris, Jacaranda, Kerria, Laburnum, Lathyrus, Leontodon, Lilium, Linum, Lotus, Lysimachia, Maratia, Medicago, Mimulus, Narcissus, Oenothera, Osmanthus, Petunia, Photinia, Physalis, Phyteuma, Potentilla, Pyracantha, Ranunculus, Rhododendron, Rosa, Rudbeckia, Senecio, Silene, Silphium, Sinapsis, Solanum tuberosum, Sorbus, Spartium, Tecoma, Torenia, Tragopogon, Trollius, Tropaeolum, Tulipa, Tussilago, Ulex, Viola or Zinnia.
39. A genetically modified plant as claimed in one of claims 30 to 36, wherein the genetically modified plant which, in comparison to the wild-type, has an increased  $\beta$ -cyclase activity in fruits is selected from the plant genera Actinophloeus, Aglaeonema, Ananas, Arbutus, Archontophoenix, Area, Aronia, Asparagus, Avocado, Attalea, Berberis, Bixia, Brachychilum, Bryonia, Caliptocalix, Capsicum, Carica, Celastrus, Citrullus, Citrus, Convallaria, Cotoneaster, Crataegus, Cucumis, Cucurbita, Cuscuta, Cycas, Cyphomandra, Dioscorea, Diospyrus, Dura, Elaeagnus, Elaeis, Erythroxylon, Euonymus, Erbse, Ficus, Fortunella, Fragaria, Gardinia, Gonocaryum, Gossypium, Guava, Guilielma, Hibiscus, Hippophaea, Iris,



Kiwi, Lathyrus, Lonicera, Luffa, Lycium, Mais, Malpighia, Mangifera, Mormodica, Murraya, Musa, Nenga, Orange, Palisota, Pandanus, Passiflora, Persea, Physalis, Prunus, Ptychandra, Punica, Pyracantha, Pyrus, Ribes, Rosa, Rubus, Sabal, Sambucus, Seaforita, Shepherdia, Solanum, Sorbus, Synaspadix, Tabernae, 5 Tamus, Taxus, Trichosanthes, Triphasia, Vaccinium, Viburnum, Vignia, Vitis or Zucchini.

40. A genetically modified plant as claimed in one of claims 30 to 36, wherein the 10 genetically modified plant which, in comparison to the wild-type, has an increased  $\beta$ -cyclase activity in tubers is *Solanum tuberosum*.

41. The use of the genetically modified plants or plant tissues as claimed in one of claims 30 to 40 as foodstuffs or feedstuffs.

15 42. The use of the genetically modified plants or plant tissues as claimed in one of claims 30 to 40 for the production of  $\beta$ -carotenoid-containing extracts or for the production of  $\beta$ -carotenoid-containing feed and food supplements.

20 43. The use of zeaxanthin-containing extracts as set forth in claim 42 for the pigmentation of animal products.